PATENT
Attorney Docket No.: 082368-001500US
Client Ref. No.: E1-A0305Y1P-US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: ITO and SAITO Application No.: 10/518,472 Filed: October 4, 2005 For: PRIMARY CULTURED ADIPOCYTES FOR GENE THERAPY Customer No.: 20350	Confirmation No. 8056 Examiner: Sajjadi, Fereydoun Ghotb Technology Center/Art Unit: 1633 DECLARATION UNDER 37 C.F.R. § 1.132 OF MASAYUKI ASO

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Commissioner for Patents
P.O. Box 1450
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Sir:

1. I am presently the President & CEO of CellGenTech., Inc. located on East Wing Shinko music plaza 2F, 2-1, Kanda Ogawamachi, Chiyoda-ku, Tokyo, Japan 101-0052.
I graduated from Hokkaido University Faculty of Pharmaceutical Science 1981.
I worked as the Product Manager for Bioproducts at Eisai Co., Ltd. From 1981-2001 and as Director of Clinical Development for hepatocyte growth factor ("HGF") gene therapy and NFκB decoy oligonucleotides at AnGes MG, Inc. from 2001-2004. I have been working as the President & CEO of CellGenTech., Inc. since 2004. CellGenTech is currently conducting research and development with gene-transfected human adipocytes for therapy of intractable diseases. Additionally, I have worked as an adjunct instructor relating to R&D of Biotechnology based drug at the requests of Chiba University and Doshisya University in Japan from 2007.

2. I am making this Declaration to provide relevant facts in support of the patentability of the subject matter claimed in the patent application. In particular, I have been asked by EISAI R&D MANAGEMENT CO., LTD., the assignee of the above-referenced application to compare the physical attributes of the multipotent adult stem cells (MASC) described in U.S. Patent No. 7,015,037 ("Furcht") to the population of preadipocytes isolated and established from adipose tissue recited in the claims of the present invention.

3. I have reviewed and am familiar with the Office Action dated October 2, 2008 ("the Office Action"), issued by the U.S. Patent and Trademark Office in the present application. I have reviewed and am familiar with the present specification, filed on December 16, 2004, and the currently pending claims.

5. My empirical determinations show that the population of primary cultured preadipocytes is materially different from MASC population disclosed in Furcht.

6. After informed consent was given, preadipocytes were isolated from seven healthy individuals according to the methods described in the specification of the above-referenced patent application. Cells were cultured by ceiling culture for seven days, and then collected, centrifuged at 300 x *g* for five minutes, washed twice with FACS buffer (2% FBS/PBS), and counted. 4.5×10^4 cells were subjected to cell-surface antigen analysis. Antibodies used for the staining of each cell-surface-antigens were CD36-FITC (Beckman Coulter; Cat#IM0766), CD44-PE (BD Pharmingen; Cat#555479), and CD146-PE (BD Pharmingen; Cat#550315). IgG1-FITC (Beckman Coulter; Cat#A07795), IgG1-PE (Beckman Coulter; Cat#A07796), and IgG2b-PE (BD Pharmingen; Cat#555743) were used as isotype controls. Antibodies were added to the cells, and then the cells were allowed to stand undisturbed for 30 minutes under dark conditions at room temperature. The cells were washed three times by centrifugation with FACS buffer at 300 x *g* for five minutes, and fixed with 200 μL of 1 % PFA/2% FBS/PBS. Analyses were carried out with FACS Calibur (BD). With respect to CD146, data was obtained for samples from five individuals.

7. The results of FACS analyses for cell-surface antigens are shown below (Fig. 1: CD44, Fig. 2: CD36, and Fig. 3: CD146 (Muc 18)). Longitudinal axis in each dot-blot indicates

the intensity of expression. Longitudinal and horizontal axis in each histogram indicates number of cells and intensity of expression, respectively. Black and red lines in each histogram indicates staining by isotype control and a labeled antibody corresponding to each antigen, respectively. The value corresponding to 1% of the maximum staining intensity obtained with the isotype control was set as a boundary value, and cells with staining intensity stronger than this boundary value were judged to be "positive". Percentages of "positive" cells for each cell-surface antigen (average) are summarized in Table 1. Fig. 4 is a photograph of cells that were obtained from human adipose tissue and cultured by ceiling culture, according to the method described in the specification of the above-referenced patent application.

Fig. 1 (CD44)

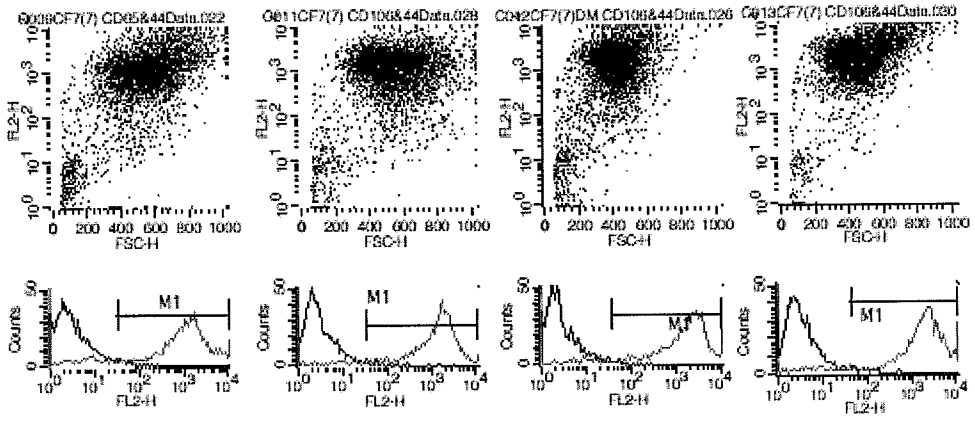


Fig. 2 (CD36)

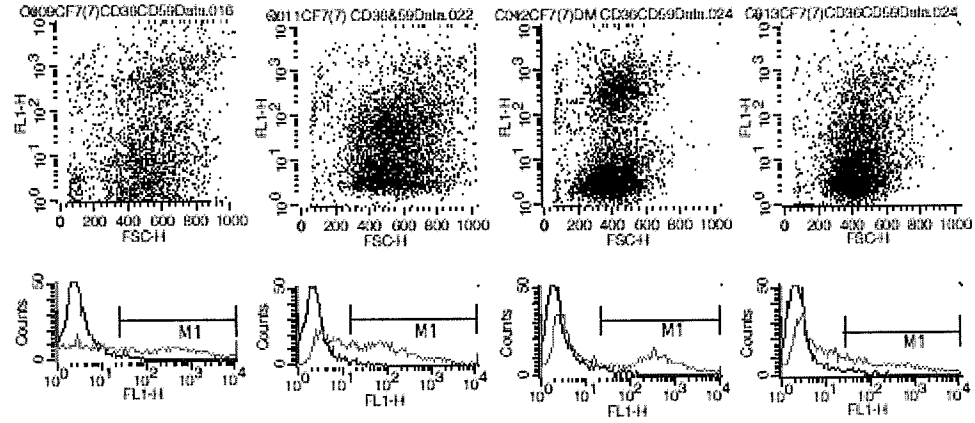


Fig. 3 (CD146(Muc18))

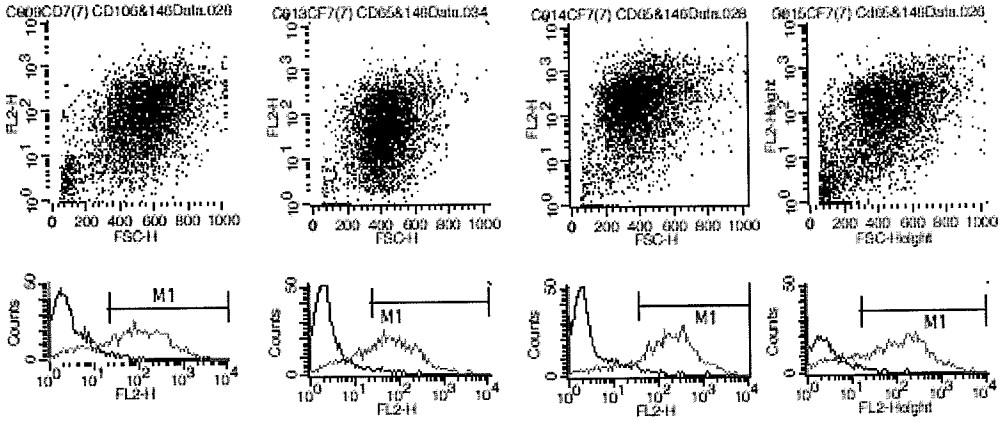
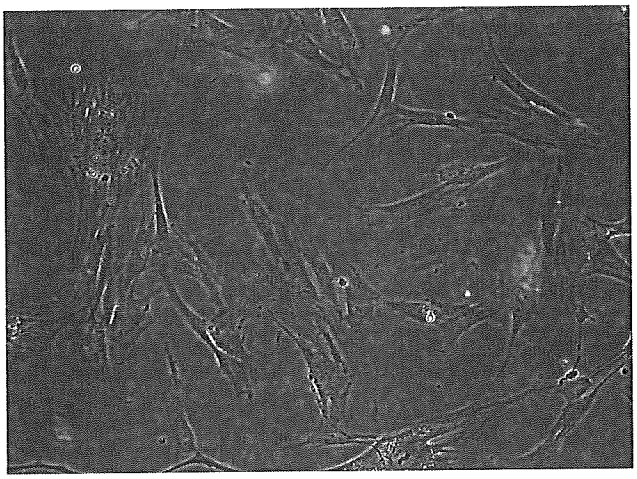


Table 1: Percentages of "positive" cells for each cell-surface antigen (average)

Percent of positive cells	CD44	CD36	CD146
Volunteer A	90.20	22.18	74.76
Volunteer B	94.64	33.56	75.42
Volunteer C	94.36	51.60	No data
Volunteer D	92.68	33.28	No data
Volunteer E	96.86	23.48	68.26
Volunteer F	94.34	21.62	84.40
Volunteer G	71.17	35.74	75.02
mean	**90.61**	**31.64**	**75.57**

Fig. 4: Cells obtained from human adipose tissue and subjected to ceiling culture



8. From the above-described results, preadipocytes isolated from human adipose tissue by the method described in the above-referenced patent application were shown to be positive for CD44, CD36, and CD146 (Muc18). This clearly indicates that the population of primary cultured preadipocytes of the above-referenced patent application are materially different from the MASC population disclosed in Furcht which is CD44(-), Muc18(-), and, especially, CD36(-). CD44 and CD146 (Muc18) were reported to be positive in adipose-derived stromal cells (see Table 6 of Exhibit A: Gronthos, *et al. Journal of Cellular Physiology* 189:54-63, 2001) and also were slightly positive after the induction of differentiation into adipose cells (Table 5 of Exhibit A); therefore, the population of preadipocytes isolated by the method described in the above-referenced patent application has properties much closer to mature adipocytes as compared to the MASCs of Furcht.

9. The fact that the population of preadipocytes obtained as described in the above-referenced patent application was CD36(+) is consistent with the conclusion that the population of preadipocytes isolated by the method described in the above-referenced patent application have a phenotype like mature adipocytes. CD36 is described in the specification of the above-referenced patent application as a possible marker for mature adipocytes (p. 5 and Exhibit B: Abumrad *et al. The Journal of Biological Chemistry* 268(24):17665-17668, 1993 (an article reporting results from rats)). In Exhibit B it was reported that the expression of CD36 was enhanced with the differentiation of adipose cells. CD36 was also shown to be positive for cells of human adipose tissue (Exhibit C: Meex *et al. The FASEB Journal*, 2005 and Exhibit D: Bonen *et al. International Journal of Obesity* 30:877-883, 2006). C/EBP responsive sequence, which is a marker of adipose cells, is present in human CD36 promoter (Exhibit E: Armesilla and Vega, *The Journal of Biological Chemistry* 269(29):18985-18991, 194) and induction of C/EBP occurs upon adipose differentiation (Exhibit F: Vasseur-Cognet and Lane, *Current Opinion in Genetics and Development* 3:238-245, 1993); therefore, CD36 is thought to be a molecule whose expression is enhanced with differentiation into adipose cells, as in the same manner with the results obtained from rats (Exhibit B). The fact that the population of preadipocytes obtained by the method of the above-referenced patent application was positive for the expression of CD36 indicates the presence of cells having a phenotype

like mature adipocytes; however, no mature adipocytes were found on the culture surface after ceiling culture (Fig. 4). This indicates that the population of preadipocytes isolated by the method described in the above-referenced patent application is (i) derived from mature adipocytes and (ii) a population that is closer to the mature adipocytes in retaining CD36 (a marker for mature adipocytes). From the above, it is considered that the cell population claimed in the above-referenced patent application possesses properties close to adipose cells and thus is suitable for use in treatment of diseases by exploiting various properties characteristic of adipocytes, as described in the above-referenced patent application.

10. I further declare that all statements made herein of my knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that any such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

11. The Declarant has nothing further to say.

Dated: Mar. 31, 2009

Masayuki Aso